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Urease-Associated Heat Shock Protein of *Helicobacter pylori*

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Helicobacter pylori **urease is an extracellular, cell-bound enzyme with a molecular weight of approximately 600,000 (600K enzyme) comprising six 66K and six 31K subunits. A 62K protein is closely associated with the** ***H. pylori*** **urease, both in crude preparations and after gel filtration; this protein can be removed from the urease by ion-exchange chromatography without inactivating the enzyme. We purified this urease-associated protein and determined its N-terminal amino acid sequence. The sequence is 80% homologous (identical plus conserved amino acid residues) to the** ***Escherichia coli*** **GroEL heat shock protein (HSP), 75% homologous to the human homolog, and 84% homologous to the HSP homolog found in species of** ***Chlamydia*****. Thus, the 62K urease-associated protein of** ***H. pylori*** **belongs to the HSP60 family of stress proteins known as chaperonins. Evidently this protein, HSP62, participates in the extracellular assembly and/or protection of the urease against inactivation in the hostile environment of the stomach.**

Helicobacter pylori is the causative agent of gastritis in humans; chronic infection with this organism contributes to formation of gastric and duodenal ulcers and possibly gastric carcinoma (1, 17, 26). *H. pylori* is found in the mucous layer of the stomach and attached to the surface of gastric epithelial cells (1, 11, 17, 26). One novel characteristic of in vitro-grown *H. pylori* is shedding of large amounts of surface-associated protein during harvesting of cells from agar culture. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses of *H. pylori* harvest fluids demonstrate many proteins, with major species having molecular weights of approximately 66,000, 62,000, 54,000, 45,000, 31,000, and 25,000. Resuspending and washing *H. pylori* cells in either water or isotonic buffered saline releases more of these same proteins. A larger yield is produced in a single step by resuspending the cells in a 0.5 or 1.0% solution of *n*-octylglucose (12, 14, 29). Earlier work showed that the 66,000-molecular-weight (66K), 62K, and 31K proteins were associated with a 600K complex having strong urease activity, but more recent work has proven that the urease is composed of 66K and 31K subunits and that the 62K protein can be removed from the complex without destroying urease activity (8, 13, 14, 18, 29).

The *H. pylori* urease is important as a putative virulence factor and as a common antigen (8, 13, 14, 17, 29, 34). Sera from *H. pylori*-infected individuals are consistently positive in an enzyme-linked immunosorbent assay which uses a combination of the 66K, 62K, and 31K proteins to capture serum immunoglobulin G antibody (8). Immunoblot studies in our and other laboratories confirm that these proteins are produced in vivo (1, 14, 17).

Another surface-associated component of *H. pylori* is a fibrillar *N*-acetylneuraminyllactose-binding hemagglutinin (13). The hemagglutinin is also readily shed under the conditions described above. We considered the possibility that the 62K protein is a component of the hemagglutinin which remains with the urease complex upon shedding or extraction.

To gain insight into the nature of the 62K protein, we purified this protein and performed an *N*-terminal amino acid analysis. The purification procedure used for this purpose differed from that previously described (14) in that higher yields of pure 62K protein were achieved.

In a typical experiment, *H. pylori* 8826 cells from 40 blood agar plates (14) were harvested into an extraction buffer consisting of 0.2% *N*-octylglucose and 1.0 M LiCl in 0.05 M Tris-Cl, pH 8.0; cells were removed by centrifugation after 20 min at room temperature. After overnight dialysis against 0.05 M Tris-Cl (pH 8.0) buffer, the extract was passed through a heparin-Sepharose (Pharmacia-LKB, Piscataway, N.J.) column. All of the proteins of interest eluted in the void volume peak fractions, which were pooled, concentrated approximately 10-fold, and eluted through a Superose-6 column (Pharmacia) with 0.025 M Tris-Cl (pH 8.0) buffer as the eluant. Fractions containing urease activity were pooled and chromatographed through a Mono-Q ion-exchange column (Pharmacia-LKB) with a 0.0 to 0.8 M NaCl gradient in the same Tris-Cl buffer. Urease eluted with approximately 0.32 M NaCl, and the 62K protein eluted with approximately 0.36 M NaCl. Figure 1 shows the results of an SDS-PAGE analysis comparing a crude extract, the purified 62K protein, and the urease. The urease prepared by this method contains two contaminating polypeptides not seen with urease purified by the DEAE method (14).

N-terminal amino analysis of the 62K protein was performed at the Baylor College of Medicine Protein Chemistry Core Facility; computer analysis of the sequence was performed at the Baylor College of Medicine Molecular Biology Information Resource Facility with the EuGene network.

From the data shown in Table 1, it is clear that the *H. pylori* 62K protein is a member of the family of HSP60 stress proteins, also known as bacterial common antigens and 60K heat shock proteins (HSPs) (5, 6, 15, 19, 20, 23, 25, 31, 32, 35).

Since the N-terminal amino acid analysis supplies information about only one portion of the molecule, we used another test, i.e., reactivity with a monoclonal antibody prepared against another HSP60 protein, to confirm the conclusion stated above. This monoclonal antibody, ML-30, was prepared against the 65K HSP common antigen of *Mycobacterium leprae* and kindly supplied by J. Ivanyi (21).

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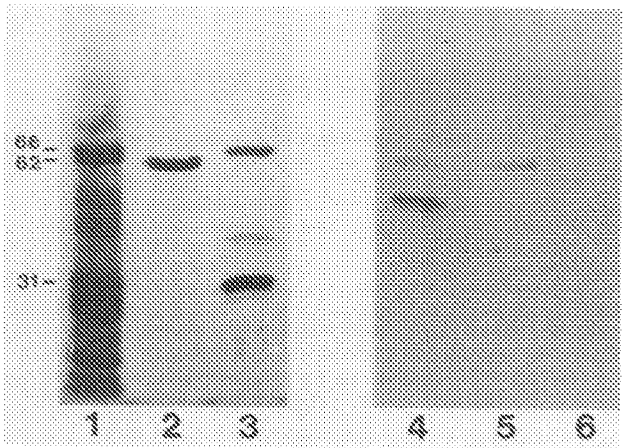

FIG. 1. SDS-PAGE of *H. pylori* crude extract (lanes 1 and 4), purified 62K urease-associated protein (lanes 2 and 5), and urease eluting from the same column (lanes 3 and 6) after staining with Coomassie blue (lanes 1 to 3) and after reaction with a 1:50 dilution of monoclonal antibody ML-30 (lanes 4 to 6). Molecular weights in thousands are indicated on the left.

Western immunoblots following polyacrylamide gel electrophoresis in SDS demonstrated that ML-30 recognizes the *H. pylori* 62K protein in purified form as well as in the crude extract (Fig. 1). Negative controls included purified urease, other extract proteins in the crude preparation, and the peroxidase-labeled conjugate alone. The only unexplained result is an additional ML-30-reactive protein in the *H. pylori* crude extract with a molecular weight of approximately 50,000 (Fig. 1).

In *Escherichia coli*, the *groEL* gene product (GroEL), the best-characterized member of the HSP60 family of proteins, is produced at low levels under optimal growth conditions but is dramatically increased by stress such as heat shock (15) and carbon starvation (22). The HSP60 proteins are a highly conserved group of proteins with closely related homologs in bacteria and other procaryotes, plant chloroplasts, and human and animal mitochondria (19), as illustrated by the examples shown in Table 1. These proteins have been termed "molecular chaperones" because they assist in posttranslational assembly, secretion, and stability of oligomeric protein structures but are not an integral part of the final oligomer (9, 12, 19, 30).

The surface location and high immunogenicity of the 62K GroEL homolog of *H. pylori*, hereafter termed HSP62, are not unusual properties despite the intracellular location of the prototype chaperonin in *E. coli* and many other bacteria. GroEL homologs have been identified as so-called common antigens of the genera *Treponema* (20), *Rickettsia* (35), *Borrelia* (5), and *Bordetella* (4) and a variety of gram-positive bacteria (25) and as genus- or group-specific *Mycobacterium* (7) and *Legionella* (32) antigens. Also, the HSP60 proteins have been implicated as bacterial survival factors in *Salmonella* (3, 16), *Chlamydia* (6), and *Mycobacterium* (7, 38) infections.

Anti-HSP immune reactions have been implicated or proven to play a role in the hypersensitivity response to *Chlamydia* infections (6, 28), in autoimmune pathologic changes in mycobacterial infections (7), in Lyme disease (5), and in Behcet's syndrome associated with *Streptococcus* infections (25). It remains to be determined whether the HSP62 of *H. pylori* causes any autoimmune pathologic changes, but there is reason to believe that this is possible. Mammalian host cells often express HSPs on their surface when stressed by infection, and these HSPs may play a part in autoimmune reactions through involvement of gamma/delta T cells (2, 24). Increased levels of the GroEL stress protein homolog in gastric epithelial cells and an increased number of intraepithelial gamma/delta T cells have been observed in patients with *H. pylori*-induced chronic gastritis (10). The fact that a major surface-associated protein of *H. pylori*, HSP62, is a GroEL homolog raises the question of whether these T cells recognize autologous HSP antigen (i.e., human P1 chaperonin [23]), by way of antigenic mimicry, as well as HSP62.

The exact function of the *H. pylori* HSP62 is yet to be proven; however, it is reasonable to speculate that HSP62 functions in the transmembrane export of the urease subunit proteins, assembly of the 600K complex after export, or

TABLE 1. N-terminal amino acid sequences of the *H. pylori* 62K protein and HSP60 homologs from a variety of sources

HSP[a]	Starting residue	44-amino-acid sequence[b]	% Homology[c]
Hp	1	A K E I K F S D S A R N L L F E G V R Q L H D A V K V T M G P R G R N V L I Q K K Y G A	100.0
Cp	3	* * + * * + + + D * * + K + H K * * + T * A + * * * * * + * * + * * + * + * + * S + * +	84.1
Ct	3	* * + * * + + + E * * + K + Q K * * + T * A + * * * * * + * * + * * + * + * + * S + * +	84.1
Ec	3	* * + + * * + + D * * V K + L R * * N V * A * * * * * * + * * + * * * * + + + * S + * *	79.6
Lp	3	* * * + + * + * D * * L Q * L A * * N A * A * * * Q * * * * * * * * * * * + + + * S * * *	77.3
Ss	24	* * R * I + + + + + * * R A * E K * + D I * A + * * A * * + * * + * * * * + + + * * + * *	77.3
Ml	2	* * T * A + D + E * * R G * E R * + N S * A * * * * * * + * * + * * * * + + + * * + * *	75.0
Hu	27	* * + + * * + A D * * A * + L + * * D L * A * * * A * * * * + * * T * + * + Q S + * +	75.0
Cb	3	* * V + * * * H E V L + A + S R * * E V * A + * * * * * + * * + * * * * + + + * S + * *	75.0
Sc	25	H * * + * * + V E + * A S * L K * * E T * A + * * A A * + * * + * * * * * * + Q P + * +	68.2
Rt	28	+ * + * V H + * Q C * K K + I * * + N V + A + * * G * * + * * + * * * * A * + Q S * * +	68.2
Ta	4	* * * * A * D + K + * A A * Q A * * E K * A + * * G * * + * * * * * * * * + + + E Y G N +	65.9

[a] Abbreviations: Hp, *H. pylori* HSP62; Cp, 57K *hypB* protein of *Chlamydia psittaci* (27); Ct, 57K *hypB* protein of *Chlamydia trachomatis* (6); Ec, *E. coli* GroEL HSP (19); Lp, *Legionella pneumophila htpB* 58K gene product, also known as 60K common antigen (32); Ss, cyanobacterial chaperonin of *Synechococcus* sp. strain PCC 7942 (37); Ml, *M. leprae* 65K HSP antigen (33); Hu, 63K human mitochondrial protein P1 (human chaperonin) (23); Cb, *Coxiella burnetii htpB* gene product (36); Sc, yeast (*Saccharomyces cerevisiae*) HSP60 (31); Rt, *Rickettsia tsutsugamushi* Sta58 major antigen (35); Ta, *Triticum aestivum* (wheat) chloroplast Rubisco subunit-binding protein alpha chain (19, 35). Amino acid sequences cited by reference were supplemented where necessary with data obtained from the EuGene data bank.

[b] +, residue conserved with respect to the *H. pylori* sequence; *, residue identical to corresponding residue in the *H. pylori* sequence.

[c] Calculated as percentage of amino acid residues either conserved or identical in comparison with the *H. pylori* sequence.

both. Given the close association between HSP62 and the urease complex, another obvious possibility is stabilization of the urease complex in the face of gastric acidity, local alkalinity caused by urease activity within the thick mucous environment, and protease attack. HSP62 could, according to our original hypothesis, chaperone the integrity of a urease-hemagglutinin complex on the *H. pylori* cell surface.

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